AMENDMENTS TO THE SPECIFICATION:

Please insert the following headings and paragraph at Page 1, line 3 of the Application-as-filed, immediately preceeding the first full paragraph:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is being filed under Rule 1.371 as a National Stage Application of International Application No. PCT/EP2003/007080 filed July 3, 2003, which claims priority to parent German Patent Application No. 102 35 348.4 filed July 3, 2002. The parent application, German Patent Application No. 102 35 348.4 is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

Please insert the following heading at Page 1, line 7 of the Application-as-filed, immediately preceeding the second full paragraph:

BACKGROUND OF THE INVENTION

Please subsitute the paragraphs beginning at Page 1 line 20 of the Application-as-filed with the following replacement paragraphs:

In addition, it is already known that patients suffering from congenital or acquired TTP are observed to lack a specific metalloprotease which cleaves VWF between the ~~peptide bonds~~ amino acids Tyr842 and Met843. This metalloprotease has recently been identified as a new member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family and designated ADAMTS-13 [[(1-3)]] Fujikawa, K., Suzuki, H., McMullen, B., Chung, D. (2001) Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. Blood 98: 1662-1666 (hereinafter "1"); Gerritsen; H.E., Robles, R., Lämmele, B., Furlan, M. (2001) Partial amino acid sequence of purified von Willebrand factor-cleaving protease Blood 98: 1654-1661 (hereinafter "2"); Levy, G.G., Nichols, W.C., Lian, E.C. Foroud, T., McClintick, J.N., McGee, B.M., Yang, A.Y., Siemieniak, DR., Stark, K.R., Gruppo, R., Sarode, R., Shurin, S.B., Chandrasekaran, V., Stabler, S.P., Sabio, H., Bouhassira, E.E., Upshaw, J.D., Ginsburg, D., Tsai, H.M. (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature 413: 488-494 (hereinafter "3").

In that which follows, the VWF-cleaving protease activity of ADAMTS-13 is simply termed ADAMTS-13 activity. ADAMTS-13 activity is normally measured by incubating a VWF sample, which has been treated with urea or guanidium hydrochloride, with dilute plasma at low ionic strength. The proteolysis is detected by means of a multimer analysis using SDS agarose gel electrophoresis or by means of fragment analysis using SDS polyacrylamide gel electrophoresis and subsequent immunoblotting, that is detecting the proteins on a cellulose membrane by means of an antigen-antibody reaction [[(4, 5)]] Furlan, M., Robles, R., Lämmle, B., (1996) Partial purification and characterisation of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood 10: 4223-4234 (hereinafter "4"); Tsai, H.M. (1996) Physiologic

Cleavage of von Willebrand factor by a Plasma Protease is dependent on its confirmation and requires Calcium ion. Blood 10: 4235-4244 (hereinafter "5"). The ADAMTS-13-catalyzed degradation of the von Willebrand factor can also be determined by measuring the collagen-binding activity of the VWF (WO-A 00/50904) or by carrying out a specific, bilateral ELISA detection [[(6)]] Obert B, Tout H, Veyradier A, Fressinaud E, Meyer D, Girma JP (1999) Estimation of the Willebrand factor-cleaving protease in plasma using monoclonal antibodies to VWF. Thromb Haemost 82: 1382-1385 (hereinafter"6"). A recombinant monomeric VWF, which has been labeled at the N terminus with a green fluorescent protein, has also recently been described for the purpose of determining the proteolysis [[(7)]] Raife TJ, Atkinsons B, Christopherson P, Jozwiak M, Montgomery RR (2001) Recombinant, truncated monomeric von Willebrand factor (VWF) for the study of VWF proteolysis. Thromb Haemost, Suppl July: Abstract#1667 (hereinafter "7").

The conventional electrophoretic methods can only be carried out in specialized research laboratories since implementing the tests requires special laboratory equipment and the requisite expertise. While the collagen-binding test (WO-A 00/50904) and the specific ELISA (6) for detecting the proteolytic activity of ADAMTS-13 simplify the determination of ADAMTS-13 activity, they can likewise only be carried out in the laboratories which have at their disposal the appropriate equipment and the necessary knowhow. Furthermore, the bilateral ELISA requires specific monoclonal antibodies which are only available in a few laboratories since they cannot be obtained commercially. The object therefore presented itself of developing a simple method for determining ADAMTS-13 activity. This novel method was to make it possible to quantify ADAMTS-13 activity in blood plasma and other body fluids (e.g. blood serum, and saliva), and other media, in a reliable and timely manner. It was to be utilizable in any routine clinical coagulation laboratory and therefore not require any special laboratory equipment, special technical knowhow or reagents which were not available commercially. Furthermore, the novel method was to permit an automation in automatic coagulation machines which was as far reaching as possible in order to make it possible

to achieve a high sample throughput at low operational cost. Since it has by now been demonstrated that low ADAMTS-13 activities can also be observed in diseases other than TTP [[(8)]] , such a method was to make it possible to differentiate between the severe ADAMTS-13 deficiency which is characteristic of TTP and mild ADAMTS-13 deficiency. Kasper, C.K. (1991) Laboratory tests for factor VIII inhibitors, their variation, significance and interpretation. Blood Coagul Fibrinolysis 2: 7-10 (hereinafter "8") An early diagnosis, and consequently a rapid initiation of plasmapheresis therapy, essentially determines the clinical course of what is frequently a life-threatening TTP episode. For this reason, the novel method was to enable ADAMTS-13 activity to be determined rapidly and as comprehensively as possible. Timely determination of the ADAMTS-13 activity is also essential because of the existence of alternative therapy options. In particular, the method was to enable an inhibitor against ADAMTS-13 to be detected rapidly, or to enable the inhibitor titer to be determined, since different treatment possibilities (e.g. rituximab or immunoadsorption) ensue from this. The method was also, in particular, to make it possible to differentiate between congenital and acquired TTP. In addition to this, timely ADAMTS-13 activity determination, which is capable of being carried out routinely, is the prerequisite for using the recombinant ADAMTS-13 which is potentially available (WO242441). The method was not only to permit timely diagnosis, and monitoring of the therapy, of TTP patients but, in addition, reliable quantification of the ADAMTS-13 activity in any arbitrary media. Since ADAMTS-13 is an important regulator of VWF, and consequently a significant factor in hemostasis, the novel method was to be applicable in diverse studies of the importance of ADAMTS-13-catalyzed proteolysis of VWF in healthy subjects and patients suffering from different diseases.

Please insert the following heading on Page 4, line 5 of the Application-as-filed, immediately preceeding the first full paragraph:

SUMMARY OF THE INVENTION

Please insert the following headings and replacement paragraph at Page 4, line 16, immediately preceeding the second full paragraph:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a is an exemplary multimer pattern illustrating the time-dependent loss of the high molecular weight VWF multimers as a reuslt of ADAMTS-13-catalyzed proteolysis.

Figure 1b is a graphical illustration of the ADAMTS-13-catalyzed loss over time of the RCo activity of the VWF substrate which has been added to the reaction medium.

Figure 2 is a graphical illustration of the ability of a given sample to aggregate platelets in the presence of ristocetin, as measured by the decrease in extinction in 90 seconds, for different dilutions of normal human plasma.

Figure 3 illustrates the results of an exemplary test using an immunoblotting method in accordance with the invention.

Figure 4 is a graphical illustration of the ADAMTS-13 activity and associated immunoblotting category for plasma samples taken from numerous patients and healthy subjects.

Figure 5 is a graphical illustration of the ADAMTS-13 activity and platelet number for an exemplary patient subjected to plasmapheresis sessions over the course of the treatment.

DETAILED DESCRIPTION OF THE INVENTION

Please substitute the paragraph beginning at Page 15 line 19 of the Application-as-filed with the following replacement paragraph:

Plasma samples were diluted 1:21 with 5 mM Tris-HCl buffer, pH 8, which contained 12.5 mM barium chloride ($BaCl_2$) and 1 mM PefaBloc SC, a serum protease inhibitor (AppliChem GmbH, Darmstadt, Germany), and then incubated at 37 °C for 5 minutes in order to activate the protease. A purified VWF (Concendre de Facteur Willebrand Humain Tres Haute Purite, LFB France) was used as substrate. The concentrate, which was free from detectable ADAMTS-13 activity, was reconstituted with water for injection to a concentration of 100 U/ml, aliquoted out and stored at -20 °C until use. Prior to the protease acting on it, the substrate was thawed, diluted, in a ratio of 1:20, with 5 M urea in 5 mM Tris-HCl, pH 8, and incubated at room temperature for 5 minutes. 100 µl of the substrate solution were then added to 210 µl of diluted plasma and the whole was left to react overnight at 37 °C. After that, the residual ristocetin cofactor activity of the added VWF substrate was determined in the reaction medium using the commercial BC von Willebrand reagent supplied by Dade Behring (Marburg, Germany). The ADAMTS-13 activity in a plasma mixture which was obtained from 80 evidently healthy adult subjects, and which was diluted 1:21, was defined as being 100 %. For calibration, serial dilutions of the plasma pool of from 1:2 to 1:32 were prepared using heat-treated plasma pools. For the heat treatment, the plasma pool was incubated at 60 °C for 30 min and then centrifuged at 13 000 rpm for 5 min (Biofuge A, Heraeus) in order to sediment protein

aggregates. The plasma which had been treated in this way did not contain any detectable ADAMTS-13 activity. The different dilutions consequently contained defined percentage quantities of ADAMTS-13 activity. The calibration curve which was obtained in this way is depicted in figure 2. The different dilutions of the normal plasma pool, which by definition contain from 200 to 0 % ADAMTS-13 activity, are plotted on the x axis. The y axis shows the ability of the corresponding sample to aggregate platelets in the presence of ristocetin, as measured by the decrease in extinction during the measurement time of 90 seconds (mE/1.5 min).

Please delete the following text beginning at Page 22, line 1 and ending at Page 23, line 13 of the Application-as-filed:

~~**Reference list**~~[[:]]

[[1.]] ~~Fujikawa, K., Suzuki, H., McMullen, B., Chung, D. (2001) Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. Blood 98: 1662-1666~~[[.]]

[[2.]] ~~Gerritsen; H.E., Robles, R., Lämmele, B., Furlan, M. (2001) Partial amino acid sequence of purified von Willebrand factor-cleaving protease Blood 98: 1654-1661~~[[.]]

[[3.]] ~~Levy, G.G., Nichols, W.C., Lian, E.C. Foroud, T., McClintick, J.N., McGee, B.M., Yang, A.Y., Siemieniak, DR., Stark, K.R., Gruppo, R., Sarode, R., Shurin, S.B., Chandrasekaran, V., Stabler, S.P., Sabio, H., Bouhassira, E.E., Upshaw, J.D., Ginsburg, D., Tsai, H.M. (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature 413: 488-494~~[[.]]

[[4.]] ~~Furlan, M. , Robles, R. , Lämmle, B., (1996) Partial purification and characterisation of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood 10: 4223-4234~~[[.]]

[[5.]] ~~Tsai, H.M. (1996) Physiologic Cleavage of von Willebrand factor by a Plasma Protease is dependent on its confirmation and requires Calcium ion. Blood 10: 4235-4244~~[[.]]

[[6.]] ~~Obert B, Tout H, Veyradier A, Fressinaud E, Meyer D, Girma JP (1999) Estimation of the Willebrand factor cleaving protease in plasma using monoclonal antibodies to VWF. Thromb Haemost 82: 1382-1385~~

[[7.]] ~~Raife TJ, Atkinsons B, Christopherson P, Jozwiak M, Montgomery RR (2 001) Recombinant, truncated monomeric von Willebrand factor (VWF) for the study of VWF proteolysis. Thromb Haemost, Suppl July: Abstract#1667~~

[[8.]] ~~Rasper, C.K. (1991) Laboratory tests for factor VIII inhibitors, their variation, significance and interpretation. Blood Coagul Fibrinolysis 2: 7-10~~[[.]]

Please enter the following Abstract beginning at Page 27, line 1 of the Application-as-filed:

ABSTRACT OF THE DISCLOSURE

The invention relates to a diagnostic method for determining the von Willebrand factor (VWF) cleaving activity of ADAMTS-13 in a test medium during which the test medium is mixed with 0.5 to 5 U/ml of a von Willebrand factor (VWF) that does not contain ADAMTS-13, and after incubation, the ADAMTS-13 activity is determined based on the drop in the VWF-mediated aggregation of thrombocytes.